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(57) SCOPE OF PATENT CLAIMS

1. A method of preparing nutritionally fortified protein raw material which is characterized in that a mixture of natural proteins for food items or feeds or (and) decomposates and amino acids is reacted transglutaminase in order to introduce amino acids to proteins or (and) their decomposates by enzymatic chemical reactions.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to a method of preparing protein raw ingredient having high nutritive value by enzyme chemically binding edible protein with amino acids using transglutaminase.

Conventionally, in order to fortify with amino acids that are nutritionally lacking in natural proteins, amino acids were simply added to proteins. However, these amino acid-added proteins are wasted largely during the processing treatment to foods and feeds, and for this reason, amino acids must be added in an appropriate amount exceeding the necessary amount by several tenths. Also, when amino acids are simply added to foods, taste, or smell that are specific to amino acids are added, and for this reason, usages as protein raw ingredients were limited. Moreover, when amino acid-added proteins are heated or frozen during the food processing and cooking processes, amino acids may be damaged or amino acids are degenerated or decomposed during the storage period.

The purpose of the present invention is to provide nutritionally fortified proteins without the aforementioned drawbacks. That is, the present invention provides a method of preparing nutritionally fortified protein raw material which is characterized in that a mixture of natural proteins for food items or feeds or (and) decomposates and amino acids is reacted with transglutaminase in order to introduce amino acids to proteins or (and) their decomposates by enzymatic chemical reactions.

According to the present invention, desired amino acid bound protein raw materials that are nutritionally fortified can be prepared by combining various types of proteins with various kinds of amino acids. In addition, amino acids are chemically bound in this invention's products. Therefore, wear during the processing treatment is minimal, no foreign taste or no foreign smell is adhered, and decomposition while being stored is minimal. Moreover, since an enzyme is used in the present invention, the binding reactions are carried out efficiently.

Raw materials used in the present invention are natural proteins for food items or feeds, and examples are plant and animal proteins such as milk casein, soybean protein, wheat gluten, beef protein, fish protein, rice protein, corn protein, etc. In addition, protein decomposates such as said protein protease decomposates can be used as raw materials.

Various kinds of amino acids can be bound with proteins, but in particular, those which are concerned with for nutritional fortification with proteins, such as methionine, cysteine, cystine, lysine, tryptophan, etc. are desirable. Amino acid derivatives such as derivatives wherein carboxyl group is methylated or ethylated can be used. However, in the case of lysine, ϵ-amino group is bound to protein so that there is need of forming a derivative.

A mixture of these proteins with amino acids is reacted with transglutaminase. In this enzymatic reaction, the following deammonization occurs between the amide group of the glutamine residue of protein or its decomposate, and the amino group of amino acids:

$$A\text{-}(CH_2)_2\text{-}CONH_2 + H_2N\text{-}R \xrightarrow{-NH_3} A\text{-}(CH_2)_2\text{-}CO\text{-}NH\text{-}R$$

(wherein A: peptide chain
R: amino acid residue)

Transglutaminase is an enzyme that catalyzes the calcium-dependent acyl transition reactions to form amide bonds between the amino group of the glutamic residue in protein or its decomposates (acyl donor) and the amino group of various kinds of compounds (acyl receptor). Transglutaminase can be obtained from the liver of guinea pigs according to Connellan's method (Connellan et al. J. Biol., Chem. 246, 1093 (1971)) and also obtained from the bovine blood.

The present invention's method is an enzymatic reaction to introduce an amino acid to protein molecules using transglutaminase. Since transglutaminase is calcium-dependent as mentioned above, the introduction reaction requires the presence of calcium. Generally, it is carried out in the coexistence of calcium chloride. If desirable, transflutaminase is activated, or for stabilization, reductants such as dithiothreitol, cycteine, glutathione, mercaptoethanol, etc. can be added.

Generally, an amino acid (2 to 10 parts), calcium chloride (0.6 parts) and transglutaminase (0.02 to 0.10 parts) are added to a solution prepared by dispersing 1 to 10 parts of protein into 1000 parts of water. The reaction is carried out at pH 6 through 8.5, at 120–40°C with stirring. The reaction time ranges from 2 to 10 hours.

After the end of the reaction, the reaction solution is treated by the ordinary method to obtain a target product. For example, after dialysis using an ultra filtration apparatus, spray dried, or proteins are precipitated by an acid or an alcohol and then the precipitate is dissolved or dispersed in water and then spray dried. The transglutaminase activity is stopped during the process of dialysis or precipitation in the aforementioned operation.

The amount of introduction of amino acids can be adjusted appropriately by changing reaction conditions such as reaction time and calcium concentration. For example, the relationships between the amount of amino acid to be introduced and the reaction time are summarized in the following Tables 1 and 2.

Table 1

Amount of lysine introduced (mols/protein 10 kg)

Reaction time (min.)	0	10	30	60	150
Amount introduced to wheat gluten	1.5	2.6	5.1	6.9	7.6

Table 2

Amount of methionine introduced (mols/proteins 10 kg)

Reaction time (min.)	0	5	10	30	60	150
Amount introduced to as1-casein	2.1	2.7	3.4	3.8	4.0	4.2
Amount introduced to β-casein	2.5	2.7	2.9	3.2	3.4	3.7
Amount introduced to soybean 7s protein	1.1	1.6	2.0	2.2	2.4	2.6
Amount introduced to soybean 11s protein	1.1	1.9	2.2	3.1	3.6	3.9

According to the present invention, variety of kinds of amino acids can be added to various kinds of proteins, and protein raw materials having high nutritional values with a good balance in the amino acid composition can be obtained. The present invention's products exhibit excellent protein efficiency and quality as shown below when compared to the conventional products prepared by simply adding amino acids.

Table 3

	Methionine content	Protein efficiency	Functional assessments
Milk protein	0.211	2.41	/
Conventional product (single addition)	0.4	3.76	Spoiled egg smell
Product of the present invention	0.4	3.93	Egg smell, but no taste

Table 4

	Lysine content	Protein efficiency	Functional assessments
Wheat protein	0.126	1.53	/
Conventional product (single addition lysine)	0.6	2.24	Amino acid smell and taste
Product of the present invention	0.6	2.41	Almost no taste

Table 5

	Methionine content	Protein efficiency	Functional assessments
Soybean protein	0.01	2.31	/
Conventional product (single addition of methionine)	0.3	3.03	Amino acid smell and taste
Product of the present invention	0.3	3.28	Almost no taste

The unit of amino acids in the aforementioned Tables 3 through 5 is amino acid g/protein nitrogen g, the protein efficiency is shown by the body weight gain/intake of protein mass. The functional assessments were conducted for total of 20 subjects including 10 males and 10 females based on the results of panel tests on taste and smell.

The present invention is explained below with reference to examples of embodiment.

Example of Embodiment 1

Wheat gluten 100 g was dispersed in 10 kg water. After adjusting at pH 7.5, calcium chloride 6 g, and dithiothreitol 15 g were added.

Lysine 30 g and transglutaminase 0.2 g were added. After heating and maintained at 37°C, the reaction was carried out for 5 hours while slowly stirring.

After dialysis using an ultra filtration apparatus, the product was spray dried and lysine-fortified wheat gluten 85 g was obtained. The fortified wheat gluten was a slightly yellowish white powder and there were no significant differences in the characteristics of the wheat gluten as the starting raw material.

The amount of lysine introduced was 83 mg per gram of wheat gluten.

Example of Embodiment 2

Milk casein 100 g was dissolved in 10 kg of water and adjusted to pH 7.5. Calcium chloride 6 g, methionine ethylester 100 g, and transglutaminase 0.3 g were added. The reaction was carried out at 37°C for 5 hours while slowly stirring. After dialysis with an ultra filtration apparatus, the product was spray dried and a white methionine-fortified milk casein 83 g was obtained. The amount of introduction of methionine was 24 mg per gram of casein.